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Drosophila E-Cadherin Regulates the Orientation of Asymmetric Cell Division in the Sensory Organ Lineage

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Summary

Background: Generation of cell-fate diversity in Metazoan depends in part on asymmetric cell divisions in which cell-fate determinants are asymmetrically distributed in the mother cell and unequally partitioned between daughter cells. The polarization of the mother cell is a prerequisite to the unequal segregation of cell-fate determinants. In the *Drosophila* bristle lineage, two distinct mechanisms are known to define the axis of polarity of the pI and pIIb cells. Frizzled (Fz) signaling regulates the planar orientation of the pI division, while Inscuteable (Insc) directs the apical-basal polarity of the pIIb cell. The orientation of the asymmetric division of the pIIa cell is identical to the one of its mother cell, the pI cell, but, in contrast, is regulated by an unknown Insc- and Fz-independent mechanism.

Results: *D*E-Cadherin-Catenin complexes are shown to localize at the cell contact between the two cells born from the asymmetric division of the pI cell. The mitotic spindle of the dividing pIIa cell rotates to line up with asymmetrically localized *D*E-Cadherin-Catenin complexes. While a complete loss of *D*E-Cadherin function disrupts the apical-basal polarity of the epithelium, both a partial loss of *D*E-Cadherin function and expression of a dominant-negative form of *D*E-Cadherin affect the orientation of the pIIa division. Furthermore, expression of dominant-negative *D*E-Cadherin also affects the position of Partner of Inscuteable (Pins) and Bazooka, two asymmetrically localized proteins known to regulate cell polarity. These results show that asymmetrically distributed Cad regulates the orientation of asymmetric cell division.

Conclusions: We describe a novel mechanism involving a specialized Cad-containing cortical region by which a daughter cell divides with the same orientation as its mother cell.

Background

During metazoan development, cell-fate diversity is in part generated via asymmetric cell divisions, in which mother cells divide to produce two daughter cells with distinct developmental potential [1]. Asymmetry often relies on the unequal segregation of cell-fate determinants. The process of unequal segregation of cell-fate determinants can be subdivided into at least three steps. First, the mother cell becomes polarized prior to mitosis. Second, cell-fate determinants localize asymmetrically to one pole of the cell to form a crescent. Third, the mitotic spindle lines up with the crescent of cell-fate determinants, thereby leading to their unequal partitioning upon cytokinesis. A key issue is to identify the molecules required to establish polarity and orient the polarity axis prior to asymmetric cell division. Studies in yeast, nematodes, and flies have shown that the polarity axis may be defined by cell-cell signaling [2], apical-basal polarity cues [3, 4], or cortical marks associated with the site of the previous cytokinesis [5].

In *Drosophila*, each sensory organ of the dorsal thorax is produced by a single precursor cell, called pI, that undergoes a stereotyped series of four asymmetric cell divisions to generate five different cells, the four cells composing a mechanosensory bristle and a glial cell [6]. At each division, distinct fates are conferred on sister cells by the asymmetric segregation of Numb, a negative regulator of Notch signaling [6, 7]. The pI cell divides within the plane of the epithelium and along the antero-posterior (ap) axis of the body to generate a posterior pIIa cell and an anterior pIIb cell that specifically inherits Numb [8]. The pIIb cell divides next along the apical-basal axis to generate an apical pIIIb cell and a small basal glial cell that inherits Numb. Then, the pIIa cell divides within the plane of the epithelium with the same orientation as its mother cell to form the socket and shaft cells [8, 9], and Numb segregates into the anterior shaft cell. Finally, the pIIIb cell divides perpendicularly to the plane of the epithelium to generate the neuron and the sheath cell.

Previous studies in flies and worms have indicated that establishment of cell polarity in mother cells involves the formation of specific domains at the cell cortex. In *Drosophila*, Bazooka (Baz), the fly homolog of *Caenorhabditis elegans* PAR-3, and Pins play a conserved role in establishing both planar and apical-basal polarity in neural precursor cells [3, 4, 10–14]. Interestingly, the localization and/or activity of Pins and Baz are regulated differently in the pI cell and in embryonic neuroblasts. In the pI cell, Disc-large (Dlg) and Pins form a complex at the anterior lateral cortex, while the conserved Baz/DmPAR-6/DaPKC complex localizes to the opposite posterior pole [10]. Frizzled (Fz) provides an initial spatial input to localize Baz and Pins at the posterior and anterior cortex, respectively. It also orients the mitotic spindle along the ap axis [8, 9, 15]. By contrast, in the neuroblast and in the pIIb cell, Inscuteable (Insc) is required to assemble and stabilize a multiprotein complex localizing at the apical pole. This complex includes Pins, Insc, Baz, DmPAR-6, and DaPKC and directs apical-basal polarity [3, 4, 9, 11–14, 16–18]. By contrast to the pI and pIIb cells, the stereotyped orientation of the pIIa division does not appear to depend on Fz or on Insc. First, in *fz* mutant pupae, the pIIa cell divides with the same orientation as the one seen for its mother cell,

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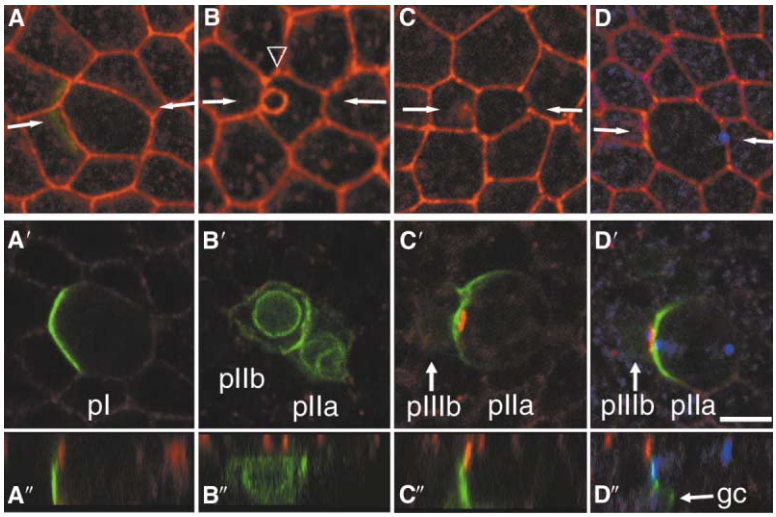

Figure 1. Cad and Arm Localize Asymmetrically in the pIIa Cell

Distribution of Cad (red in [A]–[C′′]) and Arm (red in [D] and [D′′]) in wild-type pupae. Sensory cells were identified using Pon-GFP (green in [A]–[D′′]) expressed under the control of neu^{P72}GAL4.

(A–D) Panels show apical confocal sections. (A′–D′) Panels show basal sections of the same cells.

(A′′–D′′) Panels are z-sections of these same cells (arrows in [A]–[D] indicate the z-section projection axes).

(A–A′′) In dividing pI cells, Cad remains localized to the apical cortex, while Pon-GFP forms a crescent at the lateral anterior cortex [10]. (B–B′′) Following the pI division, the apical region of the pIIb cell (arrowhead) becomes small and circular to form an apical stalk surrounded by the pIIa cell. At interphase, Pon-GFP is cortical and perinuclear ([B′]; also see [15]). During the division of the pIIa cell, (C–C′′) Cad and (D–D′′) Arm accumulate asymmetrically at the anterior cortex of pIIa to form a small patch. This cortical patch is basal to the adherens junction network formed by epidermal cells. It is, however, just apical to the (C′′ and D′′) Pon-GFP crescent (also see Movie 2) and to the (D′′) anterior centrosome (γ-tubulin is in blue in [D]–[D′′]). (D′′) Note that the posterior centrosome localizes basal to the adherens junction network. The outline of the (C′ and D′) pIIIb and glial (gc in [D′′]) cells, which are anterior and basal to the pIIa cell, respectively, is revealed by weak cortical Pon-GFP staining. Unless specified, anterior is oriented toward the left, and the midline is oriented up in all the figures. The scale bar represents 5 μm in this and all other figures.

despite the randomized orientation of the pI cell ([8, 9]; R.L.B., unpublished data). Second, Insc is not detected in the dividing pIIa cell ([9, 19]; R.L.B., unpublished data). This suggests that the orientation of the pIIa division is regulated by a novel mechanism.

Cadherins are Ca^{2+}-dependent homophilic adhesion molecules localizing at adherens cell-cell junctions (for recent reviews, see [20–22]). Cadherins also play a major role in the establishment and maintenance of apical-basal polarity in epithelial cells [23], in the formation of adhesive junctions at the synapse [24], and in the positioning of the *Drosophila* oocyte at the posterior pole of the egg chamber [25, 26]. In *Drosophila*, the major epithelial Cadherin, *D*E-Cadherin (Cad), is encoded by the *shotgun* (*shg*) gene [27, 28]. Armadillo (Arm), the fly homolog of β-Catenin, and α-Catenin directly bind to the cytoplasmic domain of Cad and connect the adherens junctions to the actin cytoskeleton [20–22]. These molecular interactions are essential for the adhesion activity of Cad. In MDCK cells, Cad-dependent cell-cell adhesion leads to the recruitment of the exocyst complex to cell-cell contacts [29]. The exocyst allows for the selective basolateral targeting of newly synthesized proteins, which is essential for the biogenesis of epithelial cell polarity. In the *Drosophila* embryo, adherens junctions have also been proposed to prevent epithelial cells from dividing asymmetrically [30].

In this study, we show that a specialized region of cell-cell contact is established between the two pI daughter cells soon after division and that this region forms a small domain at the anterior cortex of the dividing pIIa cell. The mitotic spindle rotates to line up with this Cad-rich cortical domain. Using mutant alleles of *shg* and a dominant-negative form of Cad, we show that Cad regulates the orientation of the pIIa division.

Results

Asymmetric Distribution of Adherens Junction Components

In a search for cortical markers localizing asymmetrically in the pIIa cell, we studied the distribution of the adherens junction markers Cad, Arm, and Phospho-tyrosine (P-tyr). In the pI cell, Cad, Arm, and P-tyr localize at the apical cortex, both during interphase and at mitosis (Figures 1A–1A′′; data not shown). After mitosis, these markers highlight the apical-most region of the pIIb cell that forms a tight apical stalk. As this stalk is largely engulfed by its pIIa sister cell (Figures 1B–1B′′), we suggest that the pIIb cell preferentially contacts the pIIa cell and is sorted out from neighboring epidermal cells. At this stage, Cad, Arm, and P-tyr localize apically at cell-cell contact regions (Figures 1B–1B′′; data not shown). After the division of the pIIb cell, the apical pIIIb cell contacts the pIIa cell via a stalk similar to the one seen for pIIb (data not shown). As the pIIa cell divides, this apical stalk is no more detectable, presumably due to its flattening (see below). Strikingly, Cad, Arm, and P-tyr localize asymmetrically into an anterior cortical patch in the dividing pIIa cell (Figures 1C–1D′′; data not shown; also see Movie 1 in the Supplementary Material available with this article online). This patch, which may correspond to the flattened stalk, localizes to the anterior-lateral cortex at a basal position relative to the network of adherens junctions seen in neighboring epidermal cells (Figure 1C′; also see Movie 1 in the Supplementary Material). Noticeably, this patch is found just apical to the anterior centrosome and to the cortical domain accumulating Numb, Pon (data not shown), and Pon-GFP (Figures 1C′–1D′′).

To follow the reorganization of Cad-containing complexes in this lineage in living pupa, a functional α-Cat-

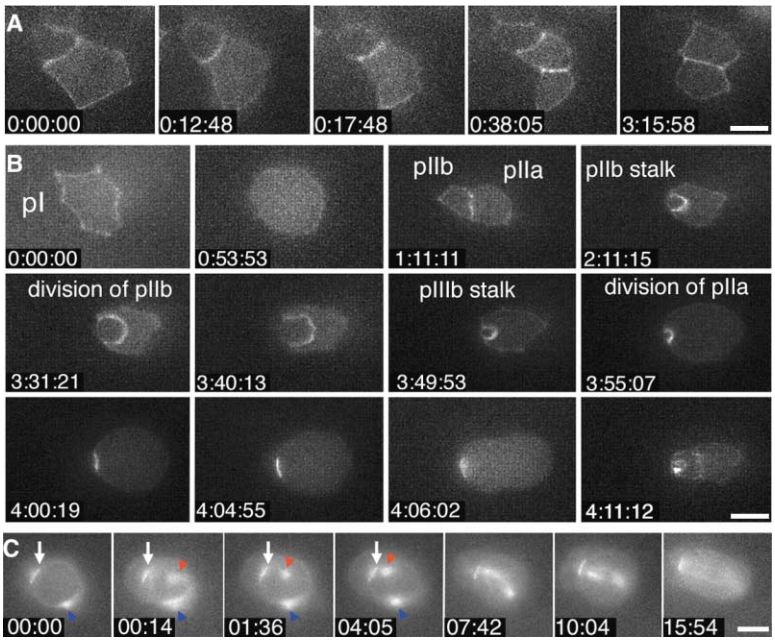

Figure 2. Dynamics of the Asymmetric Localization of α-Catenin-GFP

(A and B) Time-lapse imaging of α-Catenin-GFP distribution in dividing epidermal ([A]; see Movie 2), pI, pIIb, and pIIa ([B]; see Movie 3) cells. α-Catenin-GFP was expressed under the control of the neu^{P72}GAL4. Advantageously, a few epidermal cells also express α-Catenin-GFP, allowing a direct comparison of the localization of α-Catenin-GFP in both cell types. In epidermal cells, the cortical accumulation of α-Catenin-GFP appears to decrease in intensity during mitosis. (A) Upon cytokinesis, α-Catenin-GFP distributes evenly at the cortex in the two epidermal daughter cells. This distribution does not change over time (t = 3:15:58). (B) In the sensory organ lineage, α-Catenin-GFP distributes at the apical cortex of the pI cell prior to division (t = 0:00:00). As in epidermal cells, this cortical staining slightly decreases in intensity during mitosis (t = 0:53:53). After cytokinesis, the α-Catenin-GFP accumulates at the pIIa-pIIb junction (t = 1:11:11). This accumulation persists as the stalk of the pIIb cell forms (t = 2:11:15) and as the pIIb cell divides perpendicular to the plane of the epithelium (from t = 3:31:21 to t = 3:49:53). At the onset of the pIIa division (from t = 3:55:07 to t = 4:11:12), the pIIIb stalk flattens and the α-Catenin-GFP concentrates into a small cortical patch. This patch localizes exactly at the anterior pole of the pIIa cell (t = 4:06:02) and is redistributed apically to the pIIIb stalk after cytokinesis (t = 4:11:12).

(C) Time-lapse imaging of a dividing pIIa cell expressing both α-Catenin-GFP and Tau-GFP. The anterior accumulation of α-Catenin-GFP is indicated by the arrow. The duplicated centrosomes migrate around the nucleus of the pIIa cell to localize opposite of each other (from t = 00:00 to t = 04:05). The anterior centrosome (red arrowhead) migrates first, toward the cortical patch of α-Catenin-GFP at the end of prophase, while the posterior centrosome (blue arrowhead) begins to move as the mitotic spindle forms (from t = 04:05 to t = 07:42, see Movie 4). This suggests that molecules exerting pulling forces on centrosomes localize at the anterior cortex of the pIIa cell. Upon nuclear membrane breakdown (t = 04:05), the mitotic spindle forms and rotates to perfectly line up with the anterior patch of α-Catenin-GFP.

enin-GFP [31] was expressed in the pI cell using the neu^{P72}GAL4 line [15]. This α-Catenin-GFP colocalizes with Cad and Arm both during interphase and at mitosis in the pI cell and in its progeny cells (data not shown). In both epidermal and pI cells, α-Catenin-GFP localizes at the apical cortex. This cortical accumulation of α-Catenin-GFP is slightly reduced during mitosis (Figures 2A and 2B; see Movies 2 and 3 in the Supplementary Material). In epidermal cells, α-Catenin-GFP remains evenly distributed at the apical cortex following cell division (Figure 2A). By contrast, the morphology of the region of cell-cell contact between pIIa and pIIb dramatically changes after the pI division, as the stalk of the pIIb cell forms. Indeed, we observed that α-Catenin-GFP accumulates at the apical region of cell-cell contact between the pIIb and pIIa cells (Figure 2B). During the pIIb division, the apical stalk of the pIIb cell enlarges and rapidly reforms in the apical pIIIb cell. Then, as the pIIa cell enters mitosis, the stalk of the pIIIb cell flattens, and α-Catenin-GFP concentrates into a small cortical patch. This indicates that the anterior cortical patch corresponds to the region of cell-cell contact between the pIIa cell and the pIIIb stalk. The patch of α-Catenin-GFP persists during telophase and redistributes apically to the pIIIb stalk at the end of mitosis (Figure 2B).

To analyze how the mitotic spindle lines up with this cortical patch, the microtubule binding protein Tau-GFP was coexpressed with α-Catenin-GFP in sensory organ cells. Time-lapse imaging analysis reveals that the mitotic spindle rotates to line up with the anterior accumulation of α-Catenin-GFP (Figure 2C; see Movie 4 in the Supplementary Material). After centrosome separation, one centrosome (red arrowhead in Figure 2C) is often seen to move toward the anterior accumulation of α-Catenin-GFP during prophase (from t = 00:14 to t = 04:05 in Figure 2C). In contrast, the other centrosome (blue arrowhead in Figure 2C) moves to the opposite posterior pole only during prometaphase, as the mitotic spindle forms (from t = 4:05 to t = 07:42 in Figure 2C). This suggests that molecules localizing just basal to the α-Catenin-GFP have the ability to attract the anterior centrosome.

Together, these data indicate that, soon after the pI mitosis, cadherin-containing complexes localize at the region of cell-cell contact between the pIIa and pIIb cells. In the dividing pIIa cell, these complexes localize into a patch that coincides with the division axis of the pIIa cell. The positional information that specifies where α-Catenin-GFP accumulates at the cortex of the pIIa cell is not known (see the Discussion). One possible hypothesis is that the stalk of the pIIb cell provides such positional information. To test this hypothesis, we expressed an activated form of Notch to transform the pIIb cell into a pIIa-like cell. This change of cell fate prevented the partial delamination of the anterior cell; hence, stalk formation. Despite the lack of stalk, posterior pIIa cells were seen to divide with the same orientation as that of their mother cell (Figures 3A–3C, see Movie 5 in the Supplementary Material), along an axis defined by the anterior patch of α-Catenin-GFP. We

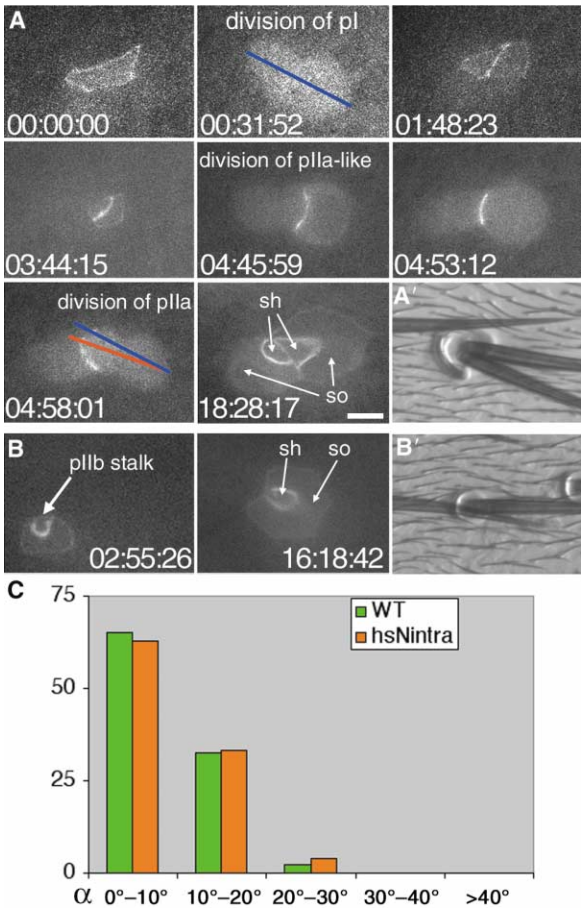

Figure 3. Formation of the pIIb Stalk Is Not Required for the Proper Orientation of the pIIa Cell Division

(A and B) Time-lapse imaging of α-Catenin-GFP driven by neu^{P72}GAL4 in (B) a control pupa or (A) after heat-induced expression of an activated form of Notch. pI cells were heat shocked (20 min at 37°C) 30 min after division (t = 00:31:52). This led the anterior cell to adopt a pIIa-like fate (see the double-socket [so] and double-shaft [sh] sense organs in Figure 3A [t = 18:28:17] and Figure 3A′; compare with Figures 3B and 3B′). In (A), α-Catenin-GFP accumulates apically between the two daughters of the pI cell, with no stalk forming in the anterior pIIa-like cell (compare t = 03:44:15 in [A] with t = 2:55:26 in [B]). Both the anterior pIIa-like cell (t = 04:45:59) and the posterior pIIa cell (t = 4:58:01) divide within the plane of the epithelium to each generate one shaft cell and one socket cell. The axis of the pIIa division (red line) is similar to that of its mother cell (blue line). (C) Plot showing that the stereotyped orientation of the pIIa cell is not affected by the pIIb-to-pIIa transformation induced by activated Notch. The angle (α) is defined by the axes of the pI and pIIa divisions measured in sensory cells expressing Pon-GFP under the control of neu^{P72}GAL4. Heat shock conditions were similar to the ones used above in (A). The angle (α) is similar in wild-type (α = 8.0° ± 4.8°; n = 46, measured using ll2B-YFP) and transformed lineages (α = 8.9° ± 6.0°; n = 27).

conclude that formation of the pIIb stalk is not required for the correct orientation of the pIIa cell.

Dominant-Negative Cad Changes the Orientation of the pIIa Division

To test the role of Cad in regulating the orientation of the pIIa division, we first adopted a dominant-negative approach and used a form of *Drosophila* Cad, dCPc3-GFP, that does not recruit Arm and α-Catenin and in which the cytoplasmic domain of Cad is replaced by a GFP tag [32]. dCPc3-GFP is expected to bind endogenous Cad and to interfere with the activity of the intracellular domain of endogenous Cad. dCPc3-GFP was expressed in the pI cell and in its progeny cells using the neu^{P72}GAL4 line [15]. As previously reported [32], dCPc3-GFP is not fully targeted to the plasma membrane and appears to stain the endoplasmic reticulum (ER). Advantageously, this ER staining outlines the position of the mitotic spindle at mitosis. Expression of dCPc3-GFP does not perturb the ap and apical-basal orientation of the pI and pIIb divisions, respectively (Figure 4A, see Movie 6 in the Supplementary Material). Following the division of the pI cell, dCPc3-GFP specifically accumulates to the region of cell-cell contact between the pIIa cell and its pIIb sister. This indicates that, under these conditions, the pIIa and pIIb cells still make specific contacts that differ from the ones established with surrounding epidermal cells.

We then examined the orientation of the pIIa division relative to the one of its mother cell by measuring the angle (α) defined by the division axes of the pI and pIIa cells (shown by blue and red lines, respectively, in Figure 4A). In wild-type lineages, the pIIa cell divides with the same orientation as its mother cell (Figure 4B). Expression of dCPc3-GFP modifies the axis of the pIIa division relative to its mother cell (Figures 4A and 4B). While only 2% of the pIIa cells divide with an α value greater than 20° in wild-type controls (n = 46), 38% of the pIIa cells expressing dCPc3-GFP show an α value greater than 20° (n = 34) (Figure 4B). Thus, expression of dCPc3-GFP affects the tight correlation between these two divisions. We also found that dCPc3-GFP does not concentrate into a cortical patch in the dividing pIIa cell, as seen for the α-Catenin-GFP, but rather distributes in a larger region (Figure 4A). This difference likely reflects a partial disorganization of the anterior patch. Indeed, we found that Arm accumulates diffusely at the anterior cortex in 56% of the pIIa cells (n = 21/37), with Numb localizing lateral to dCPc3-GFP (Figure 4E). We also analyzed the orientation of the mitotic spindle relative to the accumulation of dCPc3-GFP by video-microscopy. In 60% of the dividing pIIa cells (n = 34/56), the mitotic spindle does not line up with the anterior accumulation of dCPc3-GFP (Figures 4A and 4C). Together, these data suggest that Cad is required to recruit Arm into a small cortical patch and to line up the spindle with this patch, thereby orienting the division axis of the pIIa cell along the axis previously defined by the division of the pI cell.

Cad Regulates the Precise Orientation of the pIIa Division

To further test the role of Cad in regulating the orientation of the pIIa division, we generated mitotic clones of cells mutant for two protein null alleles, *shg*^{IG29} and *shg*^{IH}, as well as for a hypomorphic allele, *shg*$^{P34\text{-}1}$. Using Arm and Baz [11, 33] as apical markers that colocalize with adherens junctions, we show that epithelial cells mutant for null alleles of *shg* have defective apical-basal polarity (Figures 5A–5D). Two types of defects were observed

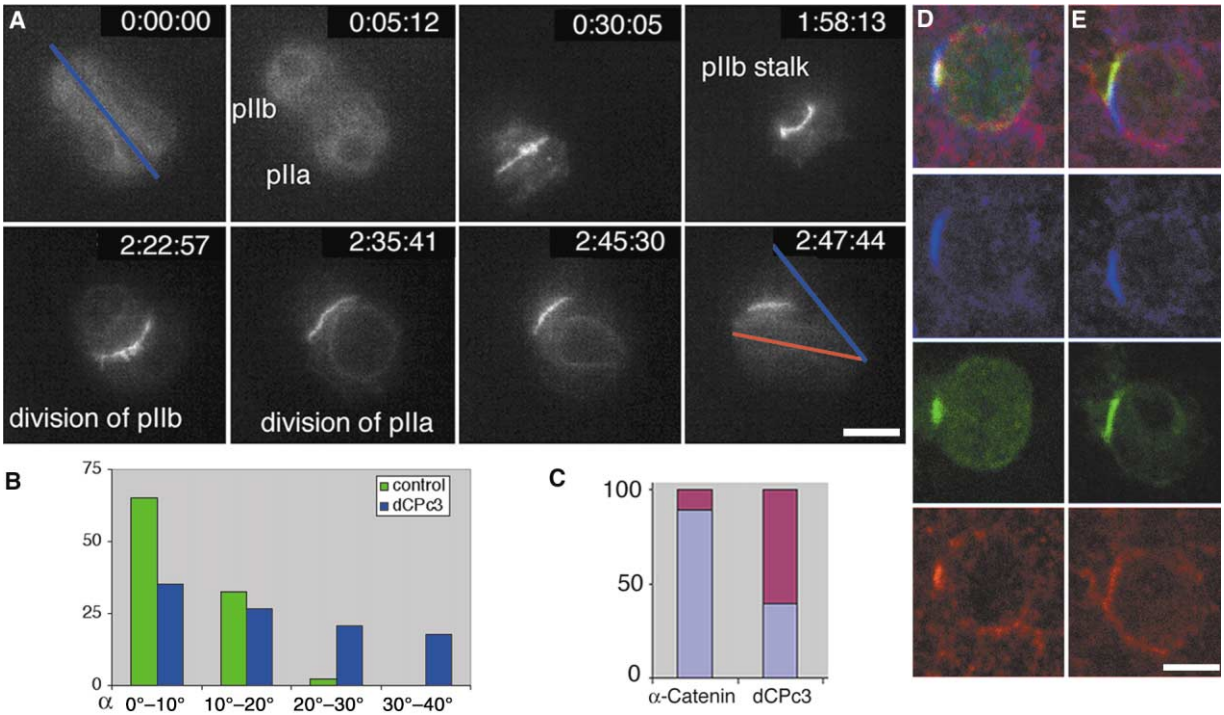

Figure 4. Dominant-Negative Cad Affects the Orientation of the pIIa Division

(A) Time-lapse imaging of sensory precursor cells expressing dCPc3-GFP. The pI (t = 0:00:00) and pIIb (t = 2:22:57) cells divide with a correct ap and apical-basal orientation. The pI division axis is indicated by a blue line. The morphology of the pIIb stalk in dCPc3-GFP-expressing pupae (t = 1:58:13) appears to be slightly different from the wild-type pIIb stalk, as it is not entirely surrounded by the pIIa cell. In the dividing pIIa cell (t = 2:45:30), the mitotic spindle often fails to align with the anterior accumulation of dCPc3-GFP. Moreover, the axis of the pIIa division (indicated by a red line) differs from the one of its mother pI cell.

(B) Orientation of the pIIa division relative to that of its mother cell pI. The angle (α), defined by the axes of the pI and pIIa divisions, is measured using H2B-YFP [15] (wild-type; green bars) ($\alpha = 8.0° \pm 4.8°$; n = 46) or dCPc3-GFP (blue bars) ($\alpha = 16.9° \pm 11.5°$; n = 34; this is significantly different from wild-type controls with $p < 0.05$). The tight correlation between these two axes is weakened upon dCPc3-GFP expression.

(C) Quantification of the misalignment of the spindle with the anterior accumulation of dCPc3-GFP. The fractions of the bars shown in blue (or purple) correspond to the percentage of the pIIa cells in which the anterior pole of the mitotic spindle colocalizes (or does not colocalize) with the anterior accumulation of α-Catenin-GFP or dCPc3-GFP.

(D and E) Confocal images showing the distribution of Arm (red) and Numb (blue) in dividing pIIa cells expressing α-Catenin-GFP (green in [D]) or dCPc3-GFP (green in [E]). The apical sections showing the localization of the GFP fusion proteins and of Arm were superimposed to the more basal section showing the crescent of Numb. Separate channels are shown below. (D) The cell-fate determinant Numb forms an anterior crescent localized just below the anterior accumulation of α-Catenin-GFP. (E) In contrast, Numb localizes both basally and laterally relative to dCPc3-GFP. In (D), Arm colocalizes with the GFP marker, apical to Numb, while (E) Arm forms a more diffuse accumulation that only partially overlaps with dCPc3-GFP and the Numb crescent.

in both *shg*^{IG29} and *shg*^{IH} mutant clones. In some clones, Arm (data not shown) and Baz are mainly found in cytoplasmic patches (Figures 5A and 5B), indicating that apical-basal polarity is lost. In some other clones, however, Arm and Baz remain mostly cortical and accumulate in the apical constrictions produced by sensory cells, suggesting that some aspects of apical-basal polarity are retained (Figures 5C and 5D). Noticeably, both types of defects can be seen within a single mutant clone (data not shown). These defects in epithelial polarity are not seen in mutant clones for a hypomorphic allele, *shg*$^{P34\text{-}1}$, that is predicted to produce a lower amount of wild-type protein [25, 26]. Consistently, in the notum, *shg*$^{P34\text{-}1}$ mutant cells have a reduced level of Cad (Figure 5E). These mutant cells have an apparently normal apical-basal polarity, with Baz colocalizing with Cad (Figure 5E). In addition, Cad and Baz accumulate at the apical region around the pIIIb stalks (Figures 5F–5I).

To study the orientation of the pIIa division in *shg*$^{P34\text{-}1}$ and *shg*^{IH} clones in which apical-basal polarity is largely maintained, we have used the mosaic analysis with a repressible cell marker (MARCM) method [34]. Briefly, a ubiquitously expressed GAL80 construct is present on the FRT chromosome homologous to that of the chromosome bearing the *shg* mutation. In addition, a UAS-Pon-GFP reporter construct activated by neu^{P72}GAL4 is also present in the strain. The GAL80 construct dominantly represses Gal4-dependent transcription. Therefore, only cells that are homozygous for the *shg* chromosome will have lost the GAL80 repressor and, hence, will express Pon-GFP in the pI cell and in its progeny (Figure 6A). Video-microscopy analysis of *shg*$^{P34\text{-}1}$ (Figure 6D) and *shg*^{IH} (Figure 6E) mutant lineages revealed that the pI and pIIb mutant cells divide with an orientation similar to the one seen in wild-type cells (Figure 6C, see movies 7–9 in the Supplementary Material). In contrast,

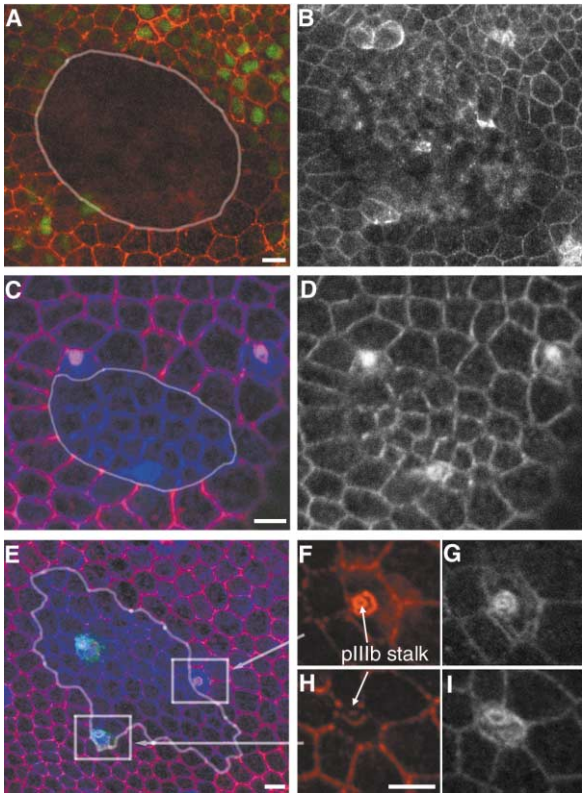

Figure 5. Loss of Apical-Basal Polarity in *shg* Mutant Cells

(A–D) Clonal analysis of shg^{IG29} function in the thoracic epithelium reveals two types of defects. *shg* clones were detected by the loss of the nls-GFP marker (green in [A]) or directly by the loss of Cad (red in [A] and [C]). (B) First, in some clones, Baz can be seen accumulating in cytoplasmic patches instead of localizing to the apical cortex. (C and D) In other clones, Baz (blue in [C], gray in [D]) appears to remain mostly cortical. In these clones, however, mutant cells have a smaller apical surface than their wild-type neighbors do. Both types of defects are seen in shg^{IG29} and shg^{IH} clones, within the same pupa or even within a single clone (data not shown). Clone boundaries are outlined in [A] and [C]. Note that these boundaries are smooth and that surrounding wild-type epidermal cells often elongate toward the mutant cells, suggesting that *shg* mutant cells are sorted out.

(E–I) A strong reduction of Cad staining (in red) is observed in cells homozygous for the hypomorphic $shg^{P34\text{-}1}$. Baz (blue in [E], gray in [G] and [I]) localizes to the apical cortex in $shg^{P34\text{-}1}$ mutant cells, suggesting that apical-basal polarity is correctly maintained in these cells. The apical stalk of (H) $shg^{P34\text{-}1}$ mutant pIIIb cells forms but contains a much reduced level of Cad when compared to (F) wild-type pIIIb cells.

the orientation of the pIIa division relative to the pI division axis is significantly more variable in *shg* mutants than in wild-type lineages (Figures 6B–6E). In wild-type controls, only 2% of the pIIa cells divide with an α value greater than 20° (n = 46). In contrast, 39% of the mutant pIIa cells divide with an α value greater than 20° (n = 23). This defect is very similar to the one observed in cells expressing dCPc3-GFP (compare Figure 6B with Figure 4B). This indicates that Cad is required to regulate the precise orientation of the pIIa division. However, a strong loss of Cad activity is not sufficient to randomize the pIIa division. This implies that additional cortical cues might control the orientation of this division in the absence of Cad.

The Position of the Cortical Domains Containing Pins and Bazooka Is Affected by Dominant-Negative Cad

In the pI cell, the asymmetric distribution of Numb at the anterior cortex has recently been shown to depend on the activity of both Pins and Baz [10, 35]. Pins and Baz localize asymmetrically at opposite poles of the pI cell. Fz signaling regulates the position of the Baz and Pins domains along the ap axis. In wild-type cells, Pins and Baz localize to the anterior and posterior cortex, respectively. In the absence of Fz signaling, Pins and Baz still localize opposite of each other but at random positions relative to the fly body axis [10]. To test, first, whether Pins and Baz may play a similar role in the pIIa cell and, second, whether Cad regulates the position of Pins and Baz, we studied the distribution of Pins and Baz in dividing pIIa cells expressing, or not, dCPc3-GFP. Pins accumulates at the anterior cortex from early prophase onward (Figures 7A and 7A′). This accumulation is concomitant with the formation of the patch of Cad (Figures 7A and 7A′) that localizes just apical to Pins. At metaphase, Pins colocalizes with Numb at the anterior cortex opposite of Baz (Figures 7B and 7B′). Expression of dominant-negative Cad does not affect the mutually exclusive distribution of Pins and Baz (Figures 7C and 7C′). However, as seen for Numb (Figure 4E), the anterior accumulation of Pins is shifted laterally relative to the cortical patch of dCPc3-GFP. This suggests that Cad regulates the precise positioning of the Pins and Baz domains.

Discussion

Three distinct mechanisms regulate the stereotyped orientation of the first three asymmetric cell divisions in the seemingly simple lineage that generates the sense organs on the *Drosophila* notum (Figure 8). In the pI cell, Fz signaling orients the mitotic spindle along the ap axis of the body [8, 15]; regulates the formation of the Dlg/Pins and Baz complexes at the anterior and posterior poles, respectively; and thereby directs the asymmetric localization of the Numb crescent to the anterior cortex [10]. By analogy to the neuroblasts, an apical Baz/Insc/Pins complex is thought to direct the apical-basal orientation of the pIIb division [3, 4, 9, 11–14, 16–18] (Figure 8). This analogy is supported by the observation that Pins, Baz, and Insc colocalize at the apical cortex of the dividing pIIb cell (R.L.B and Y.B., unpublished data). The pIIa cell divides with the same orientation as the one of its mother cell in a Fz- and Insc-independent manner. We report that, in the pIIa cell, a specific cortical domain formed at the region of cell-cell contact between the pIIb/pIIIb and pIIa cells appears to regulate the precise orientation of this division (Figure 8). Five lines of evidence support this last conclusion. First, Cad, Arm, and α-Catenin-GFP localize asymmetrically in a cortical patch at the anterior pole of the dividing pIIa cell. Second, the mitotic spindle of the pIIa cell rotates to specifically line up with this cortical domain. Third, expression

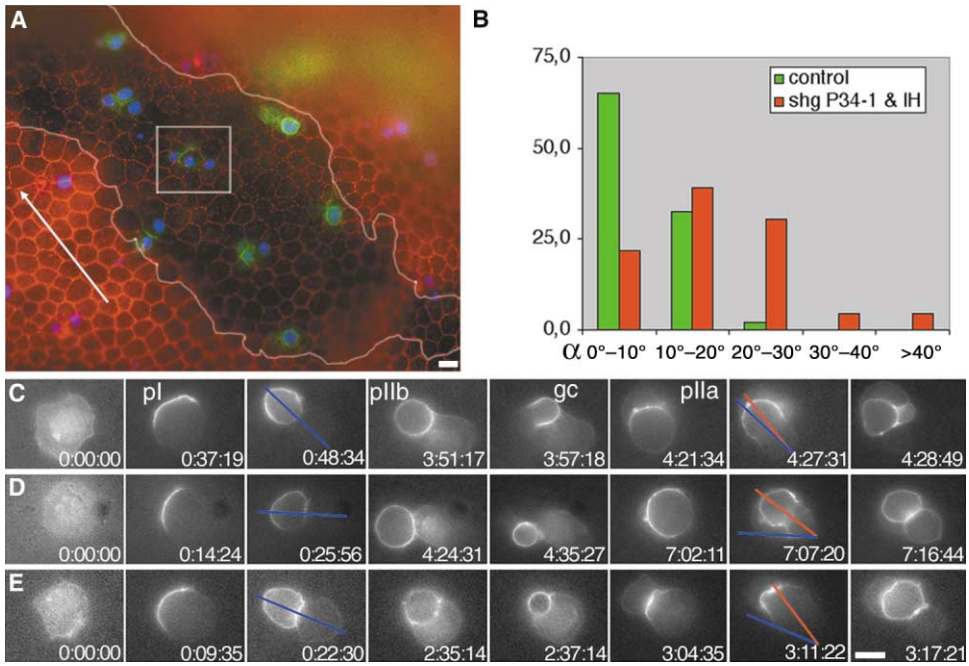

Figure 6. Cad Regulates the Orientation of the pIIa Division

(A) A strong reduction of Cad staining (in red) is observed in cells homozygous for the hypomorphic *shg*$^{P34\text{-}1}$. The pupa carrying this clone was analyzed by video-microscopy until after the mutant pIIa cells expressing Pon-GFP had divided. It was then dissected, fixed, and processed for immunofluorescence to analyze the distribution of Cad (red), Pon-GFP (green), and Cut (blue). The arrow indicates the ap axis. The square outlines the organ presented in (D).

(B) Orientation of the pIIa division relative to that of its mother cell pI. The angle (α), defined by the axes of the pI and pIIa divisions, is measured, as defined by the blue and red lines in (C–E) wild-type (green bar) and *shg*$^{P34\text{-}1}$ and *shg*^{lH} mutant lineages (red bars). The orientation of the pIIa division (red line) relative to the one of its mother cell (blue line) is more variable in *shg*$^{P34\text{-}1}$ ($\alpha = 18.2° \pm 10.8°$; n = 18) and *shg*^{lH} ($\alpha = 18.8° \pm 9.4°$ n = 5) mutant clones than in wild-type lineages ($\alpha = 8.0° \pm 4.8°$; n = 46; this is significantly different from wild-type controls with $p < 0.05$). Note that apical-basal polarity appears to be maintained in the *shg*$^{P34\text{-}1}$ and *shg*^{lH} positively marked clones analyzed here.

(C–E) Time-lapse imaging of a (C) wild-type, (D) *shg*$^{P34\text{-}1}$, and (E) *shg*^{lH} mutant pI cells expressing Pon-GFP and of its progeny cells. In (C) wild-type, (D) *shg*$^{P34\text{-}1}$, and (E) *shg*^{lH} mutant lineages, Pon-GFP forms an anterior crescent in the pI cell ([C]: t = 0:37:19; [D]: 0:14:24; [E]: 0:09:35) and unequally segregates into the anterior pIIb daughter ([C]: t = 0:48:34; [D]: 0:25:56; [E]: 0:22:30). The orientations of the divisions of these three cells are indicated by blue lines. Then, in (C) wild-type, (D) *shg*$^{P34\text{-}1}$, and (E) *shg*^{lH} mutant lineages, the pIIb cell divides perpendicularly to the plane of the epithelium, and Pon-GFP segregates into the small basal glial cell (gc; [C]: t = 3:57:18; [D]: 4:35:27; [E]: 2:37:14). In the pIIa cell, Pon-GFP forms an anterior crescent at metaphase ([C]: t = 4:21:34; [D]: 7:02:11; [E]: 3:04:35) and then segregates into the future shaft cell at anaphase-telophase ([C]: t = 4:27:31; [D]: 7:07:20; [E]: 3:11:22, and also see Movies 7, 8, and 9). The axis of the pIIa division (indicated by a red line) in the *shg*$^{P34\text{-}1}$ and *shg*^{lH} mutant lineages differs from the one of its mother pI cell.

of a dominant-negative form of Cad perturbs both the formation of this cortical domain, the orientation of the pIIa division, and the precise positioning of Pins at the anterior lateral cortex. Fourth, loss of Cad activity in clones leads to defects in the orientation of the pIIa division. Finally, we show that Pins localizes opposite of Baz in the pIIa cell along a polarity axis defined by the patch of Cad and that dominant-negative Cad affects the orientation of these two domains relative to this patch. Noticeably, a strong loss of Cad function does not randomize the orientation of the mitotic spindle or of the Pins/Baz domains. Thus, one function of Cad in the pIIa cell is to ensure precision in the orientation of the polarity axis. Although loss of Fz activity randomizes the orientation of the pI cell, Cad appears to play a role formally similar to Fz in defining the polarity axis in the pIIa cell (Figure 8). To our knowledge, this is the first evidence of a regulatory role of E-Cadherin in the orientation of asymmetric cell divisions.

Our time-lapse imaging results suggest that molecules localized at or near the anterior cortical patch capture the anterior centrosome, therefore leading to a rotation of the spindle (also see [9]). Centrosome capture is thought to depend on direct interactions between microtubule-bound proteins and specific cortical proteins. Because the anterior centrosome is located basal to the Cad-containing cortical domain, we suggest that the function of Cad is not to directly anchor the anterior centrosome, but rather to reinforce the polarized organization of the anterior cortex of the pIIa cell. In this view, the function of Cad is similar to its role in polarity establishment in cultured cells [23]. Establishment of adherens junctions in nonpolarized MDCK cells initiates the formation of distinct apical and basal-lateral plasma membrane domains by orienting the delivery of vesicles to a specific cortical site [29]. We hypothesize that *Drosophila* Cad may play a similar role in the pIIa cell. Accordingly, Cad would promote the asymmetric targeting of transport vesicles to the anterior lateral membrane and thereby determine the cortical positions of molecules attracting the anterior centrosome.

What kind of positional information might be involved

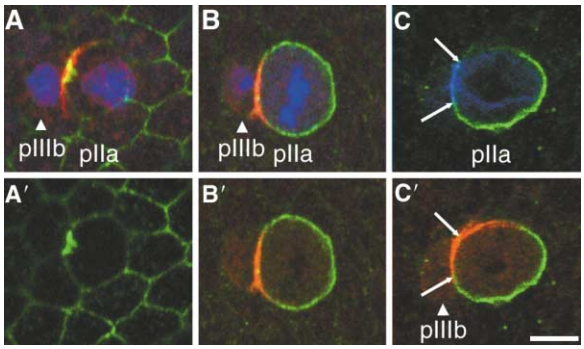

Figure 7. Dominant-Negative Cad Affects the Position of Pins and Baz

(A–B′) Distribution of Pins (red in [A]–[B′]), Cad (green in [A] and [A′]), and Baz (green in [B]–[B′]) in wild-type pupae expressing H2B-YFP (blue) under the control of neu^{P72}GAL4. Panel (A) results from the superposition of an apical confocal section showing the patch of Cad and of a more basal section showing Pins. Pins accumulates at the anterior basal cortex opposite of Baz in the dividing pIIa cell. Note that while Baz predominantly accumulates at the posterior lateral cortex at metaphase, Baz is also found at the apical anterior cortex of the pIIa cell prior to and during division (data not shown).

(C and C′) Distribution of Pins (red) and Baz (green) in a pIIa cell expressing dCPc3-GFP (blue) under the control of neu^{P72}GAL4. Pins accumulates at the anterior cortex opposite of Baz at a slightly lateral position relative to dCPc3-GFP. The arrows in (C) and (C′) delimitate the position of the dCPc3-GFP patch.

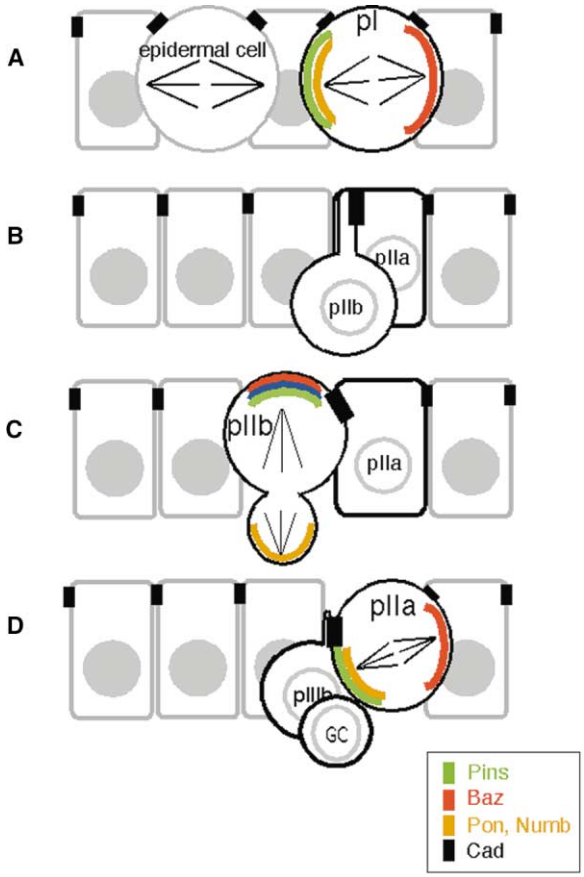

Figure 8. Three Types of Asymmetric Divisions in the Bristle Lineage of the Pupa

(A) Diagram showing the divisions of an epidermal cell (left) and of a pI cell (right). In both cells, the mitotic spindle is found below the adherens junctions (Cad in black). Epidermal cells divide with a random planar orientation. In contrast, the pI divides along the ap axis. Fz has been proposed to accumulate and signal at the posterior cortex of the pI cell [10] to orient the division of the pI cell. The Dlg/Pins (in green) and Baz (in red) complexes localize to opposite anterior and posterior cortical domains in response to Fz signaling and regulate the anterior accumulation of Numb (in orange).

(B) The pIIb cell partly delaminates and remains connected to the apical surface via an apical stalk that is engulfed by the pIIa cell. Cad localizes at the cell contact between the pIIa cell and the pIIb stalk.

(C) In the pIIb cell, Baz (in red) is thought to recruit Insc (in blue) and Pins (in green) apically. This complex would orient the division of the pIIb cell along the apical-basal axis of the epithelium, with Numb forming a basal crescent.

(D) The pIIa cell divides along an axis lining up with the anterior cortical patch of Cad. This patch stems from the region of cell contact between the pIIa and pIIb cells. Pins and Baz localize asymmetrically to opposite poles, and Cad regulates the precise orientation of these two domains. gc: glial cell.

in the localized accumulation of Cad-containing complexes in the pIIa cell? One hypothesis is that specific morphological changes in one of the two daughter cells could provide positional information for the division of the other. Accordingly, the formation of the stalk in the pIIb cell would reorganize the anterior cortex of the pIIa cell and determine the position of the cortical patch of Cad in the dividing pIIa cell. However, our analysis of the orientation of the pIIa division after inhibition of the formation of the pIIb stalk does not support this view. Alternatively, the point of cytokinesis might provide such positional information. In the budding yeast *Saccharomyces cerevisiae*, haploid cells divide in a pattern called axial in which the site of division is immediately adjacent to the previous site [5]. A small number of proteins, including the transmembrane protein BUD10, are brought to the site of the previous division [36, 37]. These proteins act as cortical marks required for the axial budding pattern. Recent evidence suggests that BUD10 directly recruits to the bud site the GDP-GTP exchange factor BUD5, which is essential for polarizing the cytoskeleton (for a review, see [38]). Here, we have shown that the pIIa cell divides with the same orientation as the one seen for its mother cell, even when the division axis of the pI cell is randomized, as in a *fz* mutant background ([8, 9]; and R.L.B., unpublished data). By analogy to the yeast axial pattern, a cortical mark localizing to the site of cytokinesis from the pI division may be used to orient the pIIa division. This mark would serve to localize Cad and Arm asymmetrically into a small cortical domain in the dividing pIIa cell. This hypothesis remains to be tested.

The polar formation of junctional complexes close to the cytokinesis site could constitute a general mechanism to regulate the orientation of an asymmetric cell division relative to the axis of the previous division. For instance, in the *Drosophila* larval brain, each neuroblast divides asymmetrically in a stem-cell mode with a fixed orientation to generate a series of ganglion mother cells (GMCs), leading to the accumulation of GMCs on one side of the neuroblast [39]. Arm and dAPC2, a *Drosophila* homolog of the Adenomatous Poliposis Coli protein, colocalize at the cell contact region between the neuro-

blast and its progeny GMCs [40]. Our study raises the possibility that, following the first round of neuroblast division, junctional complexes localizing specifically at the cell-cell contact between the neuroblast and its sister cell may orient the next neuroblast division.

Experimental Procedures

Fly Stocks

The neu^{P72}GAL4 and UAS-H2B-YFP lines are described in Bellaïche et al., 2001 [15]. Other GFP markers used were UAS-Tau-GFP [41], UAS-Pon-GFP [42], UAS-α-Catenin-GFP [43], UAS-dCPc3-GFP [32]. *shg^{IG29}* and *shg^{IH}* are putative null alleles [25, 26] that produce no detectable protein using DCAD1 and DCAD2 antibodies [44]. *shg$^{P34\text{-}1}$* is a hypomorphic allele associated with the insertion of a P element in the 5′ untranslated region of the *shg*-coding sequence [28]. Clones of cells homozygous mutant for null alleles of *shg* were studied in hs-flp/+; FRT[G13] *shg^{IG29}*(or *shg^{IH}*)/FRT[G13] Ubi-nlsGFP. Positively marked clones of *shg$^{P34\text{-}1}$* and *shg^{IH}* were generated by the MARCM method [34] in Ubx-flp/+; FRT[G13] *shg*/FRT[G13] Tub-GAL80; neu^{P72}GAL4 UAS-Pon-GFP/+ pupae and were studied by time-lapse GFP imaging. The Ubx-flp line was a kind gift of J. Knoblich (unpublished data). The hs-Nintral4A/Cyo line was used to overexpress an activated form of Notch [45].

GFP Imaging

Pupae were prepared for time-lapse analysis as previously reported [6, 15]. Briefly, pupae were fixed on two-sided tape with the notum facing up. The pupal case covering the head and the notum was removed. A glass coverslip covered with a thin layer of Voltalef 10S oil on its bottom surface was gently apposed onto the pupae with 4–5 glass coverslips on each side. Images were captured using a Micromax Camera (Princeton Instruments) connected to a DMRXA upright Leica microscope (40×, N.A. 1.25 or 63×, N.A. 1.4 oil immersion lenses) and were controlled by Metamorph Software (Universal Imaging). The exposure time was 200 ms, and images were acquired every 1–3 min. Pupae were allowed to develop to adulthood, with the exception of the pupae carrying *shg* mutant clones, which were dissected, fixed, and processed for subsequent immunostaining. Time-lapse movies were assembled using Metamorph and NIH image softwares.

Immunofluorescence

Nota were dissected and processed as previously described [46]. Primary antibodies used were rat monoclonal anti-DE-Cad (DCAD1 and DCAD2, a gift from H. Oda; 1:1000), rabbit anti-Numb (a gift from Y.-N. Jan; 1:2000), rabbit anti-γ-Tubulin (a gift from C. Gonzalez; 1:1000), mouse monoclonal anti-Arm (a gift from M. Peifer; 1:50), mouse anti-Cut (2B10, obtained from the Developmental Studies Hybridoma Bank [DSHB]; 1:1000), rabbit anti-Baz (a gift from A. Wodarz; 1:3000), and rat anti-Pins (a gift from P. J. Bryant; 1:1000). All Alexa-coupled and Cy-3/Cy-5-coupled antibodies (1:1000) were from Molecular Probes and Jackson Laboratories.

Supplementary Material

Supplementary Material including nine movies is available at http://images.cellpress.com/supmat/supmatin.htm.

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